

Original Article

Study on the Repair Effects and Mechanisms of Acanthopanax Senticosus Glycoprotein in Ultraviolet-Induced Skin Photoaging

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Abstract

Background: Photoaging is ultraviolet (UV)-induced skin aging that impairs skin health. Acanthopanax senticosus glycoprotein (As-Gp) shows antioxidant and anti-inflammatory effects, but its mechanisms in repairing UV-induced skin photoaging remain incompletely clarified. **Methods:** As-Gp was extracted and characterized. Its repair effects were assessed in long-wave ultraviolet/medium-wave ultraviolet (UVA/UVB)-irradiated human immortalized keratinocyte cell line (HaCaT) cells and rat photoaging models. Ultra-weak photon emission (UPE) was used for real-time oxidative stress monitoring. Proteomics identified core peptides, which were synthesized and validated for antioxidant activity. **Results:** As-Gp (~25 kDa) promoted HaCaT proliferation and migration, inhibited apoptosis, reduced reactive oxygen species (ROS), and restored mitochondrial potential. In rats, it reduced malondialdehyde (MDA), matrix metalloproteinase-1/3 (MMP-1/3), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6), while elevating superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and hydroxyproline (HYP). UPE confirmed its photoprotective effect. Five peptides were identified; VFDKQNGFISAAELR-(Cam) showed vitamin C-like antioxidant activity. **Conclusions:** As-Gp repairs photoaging via regulating mitochondrial homeostasis, oxidative stress, inflammation, and extracellular matrix metabolism. UPE provides a novel dynamic evaluation tool. The identified core peptide supports developing natural high-efficacy skin repair agents.

Keywords: Acanthopanax senticosus glycoprotein, antioxidant, skin photoaging, ultra-weak photon emission, peptide synthesis.

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Introduction

The skin, the largest organ of the human body, covers the entire surface and serves as a critical barrier for protection [1]. Being directly exposed to sunlight, the skin is vulnerable to excessive ultraviolet (UV) radiation, a key factor in the development of various skin conditions, such as wrinkles, desquamation, dryness, abnormal pigmentation, and even skin cancer [2,3]. Based on the wavelength of UV radiation, the UV spectrum can be divided into three parts: short-wave ultraviolet (UVC: 200–290 nm), medium-wave ultraviolet (UVB: 290–320 nm), and long-wave ultraviolet (UVA: 320–400 nm) [4]. UVC is blocked by the ozone layer, so its energy has minimal impact on

human skin. In contrast, UVA and UVB reach the Earth's surface and are the primary spectral components responsible for skin damage [5]. Studies have shown that UVA constitutes 90% of the UV radiation reaching the Earth's surface. It can penetrate deep into the dermis, inducing a burst of reactive oxygen species (ROS), triggering oxidative stress, and leading to the overexpression of matrix metalloproteinases (MMPs), ultimately resulting in collagen fiber fragmentation. UVB primarily damages the epidermis by activating inflammatory pathways (e.g., nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)) and promoting the release of pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α

(TNF- α), thereby exacerbating the progression of photoaging [6–8]. In recent years, ozone layer depletion has led to a continuous increase in surface UV intensity, contributing to a significant rise in the incidence of skin diseases associated with photoaging. This underscores the urgent need to develop efficient and safe natural intervention strategies [9,10]. However, current clinical approaches (e.g., laser therapy and chemical peeling) are often accompanied by adverse reactions such as erythema and pain, while synthetic drugs face limitations including single-targeted action and potential toxicity [11,12]. Therefore, exploring multi-targeted, low-toxicity plant-derived bioactive components has emerged as an important.

The latest advances in the treatment of UV-induced skin photoaging focus on multiple strategies, but significant limitations still exist. Clinical treatment methods mainly include invasive procedures and topical interventions: invasive techniques, such as laser resurfacing, fractional radiofrequency, and chemical peels, effectively remodel dermal collagen by ablating damaged tissue or stimulating the production of new collagen [13–15]. However, these methods often cause transient erythema, edema, and pain, with long recovery periods. Additionally, there is a risk of pigmentation or scar formation in sensitive skin. Topical synthetic drugs, including retinoid derivatives and retinoic acid, target specific pathways (such as promoting collagen synthesis or inhibiting oxidative stress) [16,17]. Their single-target action limits the efficacy against the multifactorial pathophysiology of photoaging, and some compounds suffer from poor skin permeability, instability under UV light, or potential irritancy. Emerging biologic therapies, such as growth factor-based formulations and stem cell-derived exosomes, show promise in regulating skin regeneration [18,19]. However, their high production costs, complex storage requirements, and uncertain long-term safety hinder their widespread application.

Bioactive compounds derived from natural products are increasingly being recognized as alternative options due to their multitarget effects and low toxicity [20]. Recent studies have reported the photoprotective potential of plant polyphenols (such as epigallocatechin gallate from green tea [12], resveratrol from grapes [13]), carotenoids, and polysaccharides [21]. For example, sesame glycoproteins have been shown to inhibit UV-induced MMP expression and promote collagen synthesis both *in vitro* and *in vivo* [3], while polysaccharides from *Acanthopanax senticosus* demonstrate antioxidant properties [22]. However, most studies on plant-based photoprotection agents have focused on the activity of individual components (such as polysaccharides or polyphenols), and the mechanisms of glycoproteins in photoaging repair remain insufficiently understood. Additionally, current research lacks efficient, non-invasive tools for real-time monitoring of therapeutic effects, and the identification of core bioactive motifs in complex plant glycoproteins has rarely been reported, limiting the develop-

ment of targeted, high-efficiency natural products.

Nowadays, there are numerous products for UV protection of the skin, such as sunscreens, which include both physical and chemical compositions. Physical sunscreen compositions are inorganic substances like zinc oxide and titanium dioxide, which can physically block and reflect UV rays to some extent. However, consumers often reject them due to their thick texture. Chemical sunscreen compositions are organic substances, including p-aminobenzoic acid (PABA), salicylates, and phenanthrenes, which are usually absorbed by the skin and then interact with UV rays to convert them into harmless energy; therefore, they are called UV absorbers. However, these ingredients can be irritating and allergenic to the skin due to the chemical reactions that occur on the skin. Therefore, prevention alone is insufficient to solve UV-induced skin problems; both prevention and repair should be carried out simultaneously. More recent attention has focused on natural antioxidant components found in plants. Active ingredients such as polyphenols, carotenoids, plant saponins, and polysaccharides have been studied for their ability to attenuate and restore, to varying degrees, the skin erythema and photoaging induced by UV exposure.

Acanthopanax senticosus is a plant of the genus *Acanthopanax* in the family Araliaceae. In Traditional Chinese Medicine (TCM), it is believed to have the efficacy of nourishing and strengthening the body, as well as reinforcing healthy qi to combat aging. Its *Acanthopanax senticosus* glycoprotein (As-Gp) has attracted considerable attention due to the synergistic effects of both polysaccharides and proteins. Glycoproteins are known to be one of the effective components in *Acanthopanax senticosus*, and plant glycoproteins exhibit diverse biological effects [23]. *Acanthopanax senticosus* has been shown to scavenge various free radicals, such as hydroxyl radicals, superoxide anions, and hydrogen peroxide [24,25]. Previous studies have demonstrated that As-Gp exerts antioxidant and anti-inflammatory effects by scavenging free radicals and inhibiting inflammatory pathways. However, the mechanism of action of As-Gp in UV-induced skin photodamage repair remains unclear, particularly the correlation between its structural characteristics and bioactive components, an area that has not been systematically investigated. Based on this, this study focuses on the following scientific questions: Can As-Gp repair photoaging damage by regulating the imbalance of the oxidation-antioxidant system? Are its anti-inflammatory and matrix-protective effects related to the inhibition of MMPs and inflammatory factors? Additionally, can key bioactive peptides be screened and their functions validated using proteomic technologies? To address these questions, this study optimized the extraction process to obtain high-purity As-Gp and combined spectroscopic analysis and Western blotting to characterize its O-glycopeptide bond structure. Using UVA/UVB-induced human immortalized keratinocyte cell

line (HaCaT) cells and a rat photoaging model, the regulatory effects of As-Gp on oxidative stress markers (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA)), inflammatory cytokines (IL-1 β , TNF- α), and matrix metabolism (matrix metalloproteinase-1 (MMP-1), hydroxyproline (HYP)) were systematically evaluated. Furthermore, key functional proteins were identified through proteomics, and characteristic peptides were synthesized to validate their antioxidant activity.

This study systematically elucidated the molecular mechanisms by which As-Gp repairs UV-induced skin photoaging through multi-target synergistic effects. Additionally, for the first time, it applied biological ultra-weak photon emission (UPE) technology to assess the dynamic changes in skin oxidative stress induced by UV radiation. This approach enables non-invasive, real-time monitoring of skin photodamage and provides an efficient tool for drug screening. Furthermore, the potent antioxidant function of the core bioactive peptide VFDDQNGFISAAELR-(Cam) was identified. These findings not only provide a scientific foundation for developing TCM glycoprotein resources and creating skin photoprotective products but also have significant implications for promoting the translational application of plant pharmacology in dermatology.

Materials and Methods

Materials and Reagents

The whole *Acanthopanax senticosus* medicinal materials were collected from Jiangyuan District, Changbai County, and Jingyu County in Jilin Province. They were identified as the roots and rhizomes of *Acanthopanax senticosus*, a plant of the Araliaceae family, by Professor Li Yiping from the School of Pharmacy, Changchun University of Chinese Medicine. The HaCaT was purchased from Guangzhou Yuanzheng Biotechnology Co., Ltd., for use as human-derived cells. Forty female specific pathogen-free (SPF) grade Sprague-Dawley (SD) rats, weighing 200 ± 20 g and aged 6–8 weeks, were obtained from Changchun Yisi Experimental Animal Technology Co., Ltd. (Certificate No.: SCXK(Ji)-2020-0002). The HaCaT cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated at 37°C in a 5% CO₂ incubator.

Optimization of the Extraction Process for As-Gp

Five grams of *Acanthopanax senticosus* powder were extracted using pH 8.0 phosphate-buffered saline (PBS) buffer (1:16, w/v) as the extraction solvent under constant-temperature oscillation at 70°C (180 rpm, 12 hours $\times$ 2). The extraction solution was then centrifuged (8000 $\times$ g, 15 minutes), and the supernatant was vacuum-dried to obtain the crude extract. The crude extract underwent alcohol precipitation, protein removal by the Sevage method (chloroform:n-butanol = 3:1, sample:mixture = 3:1), and

dialysis purification. Finally, the purified product was freeze-dried to yield purified As-Gp, and its purity was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Structural Characterization of As-Gp

Glycoproteins are proteins that contain sugar chains [26]. Pan-O-linked N-acetylglucosamine modification (Pan-O-GlcNAc) antibodies were used to detect the O-GlcNAc modification sites of As-Gp via Western blot [27]. Its physical properties were characterized by analyzing appearance, pH, and UV absorption spectroscopy, and its content was determined using the bicinchoninic acid (BCA) assay.

Determination of UV Spectrum

The change in absorbance at 240 nm was detected using the β -elimination method, and the correlation of glycopeptide bond types was analyzed by combining it with UV spectral scanning ranging from 200 to 350 nm [28].

Determination of Infrared (IR) Spectrum

Take 1–2 mg of As-Gp, mix it with potassium bromide, grind the mixture, press it into a pellet, and analyze it using IR spectroscopy ($400\text{--}4000\text{ cm}^{-1}$) to characterize its functional group structure.

Monosaccharide Composition Analysis of As-Gp

The As-Gp (10 mg) was hydrolyzed with trifluoroacetic acid (TFA) in a sealed ampoule for 2 hours. After cooling, the mixture was filtered and dried under reduced pressure. The residue was derivatized with hydroxylamine hydrochloride/pyridine for 30 minutes, followed by treatment with acetic anhydride for 30 minutes. The mixture was then extracted with chloroform and dried under vacuum for 24 hours. Sugar cyanohydrin acetate derivatives of standard monosaccharides were prepared using the same method and analyzed by gas chromatography (GC) [29].

Amino Acid Composition Analysis of As-Gp

The As-Gp was mixed with 18 mL of hydrochloric acid solution in a hydrolysis tube, sealed under vacuum, and hydrolyzed at 110°C for 24 hours. After cooling, the mixture was diluted to 50 mL, filtered, and the filtrate was dried under vacuum prior to amino acid analysis [30].

The Effect of As-Gp on Skin Photoaging in Rats

Female SD rats were acclimatized for one week, after which a $4 \times 4\text{ cm}^2$ area on the dorsal skin was depilated. The irradiation conditions were determined according to the minimal erythema dose (MED, 35.63 J/cm^2), with the irradiation distance set at 20 cm. Combined UVA and UVB radiation was used to simulate sunlight exposure. The irradiation protocol was as follows: 0.5 h on day 1; 1 h on days 2–4; 2 h on days 5–10; and 3 h on days 11–21. Ir-

radiation was continued until desquamation, sunburn-like lesions, or even ulceration appeared in the modeled dorsal skin area, indicating successful establishment of the photoaging model. [31]. The rats were divided into six groups: one group that did not receive any irradiation, one group for modeling purposes, and three experimental groups treated with As-Gp at low, medium, and high doses (5, 10, and 20 mg/mL), along with VE treatment. The detailed grouping is as follows: 1. control group: Without UV exposure or drug treatment. 2. Model group: UVA/UVB irradiation without drug treatment. 3. VE group: UVA + UVB irradiation, with the dorsal skin uniformly coated with 10 mg/mL VE solution once daily. 4. As-Gp L group: UVA + UVB irradiation, with the dorsal skin uniformly coated with 5 mg/mL As-Gp solution once daily. 5. As-Gp M group: UVA + UVB irradiation, with the dorsal skin uniformly coated with 10 mg/mL As-Gp solution once daily. 6. As-Gp H group: UVA + UVB irradiation, with the dorsal skin uniformly coated with 20 mg/mL As-Gp solution once daily.

Gradually increasing UV irradiation was applied until skin damage was established. After intervention, skin thickness and repair status were dynamically observed (Fig. 3A). Back skin samples were paraffin-embedded, and oxidative stress markers (MDA, GSH-Px, SOD), matrix components (HYP, MMP-1/-3), and inflammatory cytokines (IL-1 β , interleukin-6 (IL-6), TNF- α) were detected. Hematoxylin-eosin (H&E) and aldehyde fuchsin double staining were used to evaluate epidermal/dermal tissue morphology and the collagen-elastin fiber network. Additionally, HE staining was conducted on the heart, liver, spleen, lungs, and kidneys for histological examination.

Detection of UPE From Photodamaged Skin in Rats

The test rats and the animal ventilated anesthesia machine were placed in the operating darkroom in advance. After 30 minutes of dark adaptation, the rats were anesthetized with 2.5% isoflurane (0.5 L/min) for induction, and anesthesia was then maintained with 1.5% isoflurane (0.5 L/min) while the rats were positioned in the prone position. Using Andor Solis 4.3 software, the modeling/drug administration site on the rats' backs was identified via the Single program, with the lens positioned 17 cm away from the skin. After fixation, UPE images were continuously acquired using the Accumulate program, with each session lasting 1 minute for a total of 15 minutes. Photon intensity was simultaneously recorded to dynamically monitor the recovery status of skin oxidative stress following long-term irradiation.

As-Gp Promotes HaCaT Cell Proliferation

HaCaT cells were seeded into a 96-well plate at a density of 5×10^3 cells per well, with a cell-free blank control group receiving 100 μ L of culture medium without cells. After 24 hours, the medium was discarded, and me-

dia containing As-Gp at concentrations of 0, 5, 10, 50, 100, 200, and 400 μ g/mL were added to the wells (6 replicates per group). The negative control group was treated with the same volume of medium. After 48 hours, 20 μ L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL; BCCC0464V, Sigma-Aldrich, St. Louis, MO, USA) was added to each well, followed by incubation for 4 hours. The medium was then removed, 150 μ L of dimethyl sulfoxide (DMSO) (STBH7424V, Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and the optical density (OD) values of each well were measured at a wavelength of 490 nm. The cell proliferation calculation formula is as follows:

$$\text{Cell viability (\%)} = \left\{ \frac{OD_E - OD_B}{OD_N - OD_B} - 1 \right\} \times 100\% \quad (1)$$

Wound Healing Test

A scratch assay was used to assess the ability of HaCaT cells to migrate in a two-dimensional plane. Briefly, HaCaT cells were seeded into a 6-well plate. After adhesion, scratches were made in the cell monolayer, and the medium was replaced with culture medium containing different concentrations of As-Gp. The cells were then incubated for 24 hours, and the scratch areas were observed under an inverted microscope and analyzed using ImageJ 1.54f (National Institutes of Health, Bethesda, MD, USA). The scratch healing rate was calculated using the following formula:

$$\text{Scratch healing rate (\%)} = \frac{(S_0 - S_{24})}{S_0} \times 100\% \quad (2)$$

Transwell Assay

The migratory potential of HaCaT cells in three-dimensional space was evaluated using a Transwell assay following treatment with As-Gp. Briefly, 4×10^5 HaCaT cells were co-seeded with the drug in the upper chamber of a Transwell insert (12-well plate format) using serum-free medium (400 μ L). Subsequently, 1.2 mL of serum-free medium was added to the lower chamber. The cells were cultured for 48 hours. After incubation, the insert was removed, washed twice with PBS, and the cells remaining in the upper chamber were gently scraped off with a cotton swab. The insert was then dried with filter paper. Both the upper and lower chambers were fixed separately with 1 mL of 4% paraformaldehyde for 30 minutes, washed with PBS, and dried again. Next, the chambers were stained with 1 mL of 0.1% crystal violet for 30 minutes. Migrated cells were photographed under a microscope, and the number of migrated cells was quantified using ImageJ software.

Flow Cytometry Analysis of Cell Apoptosis and Mitochondrial Membrane Potential

Flow cytometry was used to further assess cellular apoptosis ratios with an annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (7401432, BD Biosciences, San Jose, CA, USA). FITC-conjugated annexin V served as a probe for the detection of early-stage apoptosis in cells. Following As-Gp treatment and UVB irradiation, HaCaT cells from all groups were collected and incubated with annexin V-FITC/PI for 15 min before adding 400 μ L of $1 \times$ binding buffer. The cells were then thoroughly mixed and placed on ice. Flow cytometric analysis was performed to evaluate apoptotic outcomes.

To evaluate the effects of As-Gp on mitochondrial function, the mitochondrial membrane potential ($\Delta\psi$ m) was assessed using 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) staining combined with flow cytometry. HaCaT cells were seeded into 6-well plates at a density of 1×10^6 cells per well and incubated for 24 hours. The cells were then treated with various concentrations of As-Gp and exposed to UV irradiation. After UV exposure, As-Gp treatment continued for an additional 6 hours. Following treatment, the cells were harvested using EDTA-free trypsin, washed twice with cold PBS, and resuspended in JC-1 working solution (10 μ g/mL) according to the manufacturer's instructions (Beyotime, C2006). The cell suspension was incubated at 37 °C for 20 minutes in the dark. After incubation, the cells were centrifuged at $600 \times g$ for 5 minutes and washed once with JC-1 staining buffer. Flow cytometry was performed using a flow cytometer (e.g., Beckman A00-1-1102). JC-1 monomers (indicating depolarized mitochondria) were detected in the FITC channel (green fluorescence), while JC-1 aggregates (indicating polarized mitochondria) were detected in the PE channel (red fluorescence). The ratio of red to green fluorescence was calculated to assess changes in mitochondrial membrane potential.

Cellular ROS Assay

HaCaT cells were seeded into a 6-well plate at a density of 5×10^5 cells per well. When the cells reached approximately 70% confluence, they were switched to serum-free medium for synchronization and treated for 12 hours. UVA and UVB irradiation were applied to stimulate the cells and establish a pathological model. After treatment, cells were collected and washed twice with PBS. ROS detection was performed according to the protocol of the Beyotime S0033S kit, followed by analysis using flow cytometry (Beckman A00-1-1102) with the FITC channel.

Proteomic Analysis of As-Gp and Validation of Its Active Components

Target identification: The 25 kDa band from SDS-PAGE was subjected to destaining, dehydration, reduc-

tion, and alkylation. After incubation with an NH_4HCO_3 suspension on ice for 1 hour, the sample was digested overnight with trypsin at 37 °C. The resulting peptides were lyophilized and analyzed by LC-MS/MS. Chromatographic gradient: The gradient was set as follows: 6–35% B from 0 to 22 min, 35–80% B from 22 to 26 min, and 80% B from 26 to 30 min, with a flow rate of 550 nL/min. Peptide synthesis: Peptide synthesis was conducted using a 12-channel semi-automatic peptide synthesizer (Prelude X, Gyros Protein Technologies, Scottsdale, AZ, USA). The synthesis protocol for *in vitro* synthesized peptides 1–5 is provided in **Supplementary Table 1**.

Identification of the Target Peptide of As-Gp by in vitro Synthesis

The peptide was characterized using HPLC-MS. Chromatographic conditions: mobile phase A (0.1% TFA in acetonitrile) and mobile phase B (0.1% TFA in water), with gradient elution (15% to 40% B over 0–20 min; flow rate: 1 mL/min). Electrospray ionization (ESI) source parameters: desolvation temperature 450 °C; cone voltage 15–30 V; capillary voltage $\pm$ 2.5–3.5 kV. Tandem mass spectra were analyzed using PD 2.4. Physicochemical properties, including purity, molecular weight, and solubility, were simultaneously determined.

Validation and Comparison of in vitro Antioxidant Activity Between As-Gp and Synthetic Peptides

The antioxidant and anti-photoaging effects of As-Gp and five synthetic peptides (peptide GVGMPVDHPHGGGEGR (GVG), peptide EAYPGDVFFYLHSR (EAY), peptide VFDKQNGFISAAELR (VFD), peptide AVVVHGDPPDDL (AVV), peptide EADVDGDGQINY-EEFVK (EAD)) were systematically evaluated through three pathways: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, SOD activity, and total reducing power. Experiments were conducted according to the instructions of the corresponding kits.

HaCaT cells were cultured in confocal dishes at a density of 5×10^5 cells per well. When the cells reached 70% confluence, they were incubated in serum-free medium for 12 hours to induce starvation. UVA irradiation was then applied to establish the model. Subsequently, each well was treated with vitamin E (VE), As-Gp, or VFD for 24 hours. Experiments were performed according to the instructions of the ROS detection kit, and images were captured using confocal microscopy.

The in vivo Anti-Photoaging Comparison Between As-Gp and Synthetic polypeptide

Using UVA/UVB simulation of sunlight to establish a rat skin photoaging model, we observe the recovery of the rat skin after photoaging and perform HE and aldehyde fuchsin staining of the tissue sections to deeply investigate the morphological changes in the skin tissue. Further, we

explore the therapeutic effects of As-Gp and synthetic peptides on rat skin photoaging through oxidative stress.

Statistical Analysis

All experiments were performed at least in triplicate. Data are presented as the mean $\pm$ standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test. Tukey's test was used for pairwise comparisons between groups. Statistical analysis and visualization were performed using GraphPad Prism 9 software (GraphPad Software, LLC, Boston, MA, USA). Statistical significance was set at $p < 0.05$.

Results

Structure and Physicochemical Properties of As-Gp

Numbered lists can be added as follows: Extraction process of As-Gp (Fig. 1A): The crude extract was subjected to alcohol precipitation, deproteinization using the Sevage method, and purification by dialysis, yielding a brownish-yellow lyophilized powder (protein content: 32.12%, neutral sugar content: 16.27%, acidic sugar content: 24.77%, Fig. 1B). The product was readily soluble in water (pH 7.3) and exhibited a characteristic UV absorption peak at 285 nm. SDS-PAGE revealed a major band at approximately 25 kDa (Fig. 1C). NaOH treatment caused a shift in the absorption peak to 240 nm (Fig. 1D), confirming the presence of O-glycopeptide bonds (formed by the alkaline hydrolysis of threonine/serine) [32].

Protein glycosylation is generally categorized into three types: N-glycosylation, O-glycosylation, and GPI-anchored glycosylation [33]. Among these types, the hydroxyl groups on serine or threonine residues are more prone to O-glycosylation [34]. An important modification in O-glycosylation is the O-GlcNAc [35]. Western blot analysis confirmed the presence of O-GlcNAc modification sites (Fig. 1E), with the main band of the modified protein showing a molecular weight of approximately 25 kDa (consistent with the SDS-PAGE results). Glycosylation refers to the process by which glycosyltransferases transfer sugars to proteins, forming glycosidic bonds and converting proteins into glycoproteins [36]. This finding supports the conclusion about the type of glycopeptide bond identified.

The IR spectrum (Fig. 1F) of As-Gp exhibited characteristic absorption peaks at 3300 cm^{-1} (O-H/N-H stretching vibration), 1600 cm^{-1} (amide bond), and 1100 cm^{-1} (pyranose C-O bond). The monosaccharide composition and content of As-Gp were investigated using GC. The gas chromatographic analysis of the standards and samples is shown in **Supplementary Fig. 1** and **Supplementary Fig. 2**. By calculating the retention time and characteristic peak areas of the standard monosaccharides and samples, it was determined that the main monosaccharides in As-Gp include glucose, arabinose, and xylose (Table 1).

Amino acids are the fundamental building blocks of

Table 1. Analysis of the monosaccharides content of As-Gp.

Carbohydrate	Content (%)
Fucose	0.15
Rhamnose	1.47
Arabinose Ara	3.77
Galactose	12.56
Glucose	70.28
Ribose	0.58
Fructose	5.01
Xylose	3.42
Mannose	1.76
Galacturonic acid	0.59
Guluronic acid	0.18

proteins and essential nutrients in living organisms, playing an indispensable role in biological activities [37]. Amino acid composition analysis (Table 2) identified 18 amino acids, including all 8 essential amino acids, with aspartic acid being the most abundant. These findings confirm that this is a high-quality plant glycoprotein.

Table 2. Analysis of the amino acid content of As-Gp.

Amino acid name	Content (mg/g of As-Gp)
Aspartic acid	6.31
Threonine	1.10
Serine	2.31
Glutamic acid	5.90
Glycine	1.55
Alanine	3.02
Valine	1.14
Isoleucine	0.99
Leucine	2.54
Tyrosine	1.05
Phenylalanine	1.35
Histidine	0.42
Arginine	4.65
Lysine	1.41
Cystine	0.46
Methionine	0.38
Proline	2.74
Tryptophan	1.62

Therapeutic Repair of Skin Photoaging in Rats Mediated by As-Gp

After successful model establishment, ultra-weak luminescence detection was performed on the rats during the drug administration period, and changes in skin condition were photographed and recorded, including measurements of skin thickness and recovery status (Fig. 2A). On day 7 of drug administration, significant dose-dependent improvements in skin phenotypes were observed across all groups (Fig. 2B). The model group exhibited typical photoaging

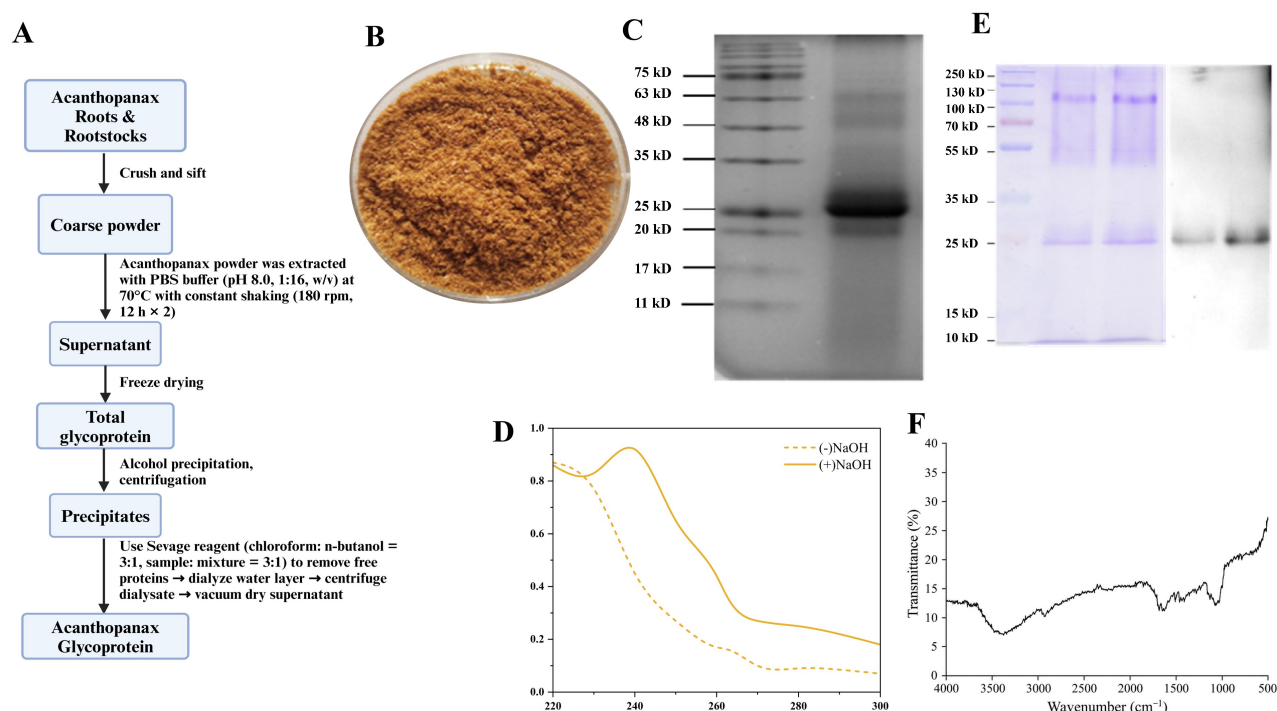


Fig. 1. Structural and physicochemical properties of As-Gp. (A) Extraction process flowchart. (B) Morphology of the lyophilized product. (C) SDS-PAGE analysis showing a predominant band at approximately 25 kDa. (D) UV characteristic absorption peak at 285 nm. (E) Western blot detection of O-GlcNAc glycosylation modification sites. (F) FTIR spectroscopy for functional group identification. Abbreviations: As-Gp, Acanthopanax senticosus glycoprotein; PBS, phosphate-buffered saline.

characteristics, including leathery thickening and epidermal damage, while the medium-dose ($p < 0.001$) and high-dose ($p < 0.0001$) As-Gp groups (10–20 mg/mL) showed epidermal structural reconstruction, manifested as a significant reduction in skin thickness (Fig. 2E), hair regeneration, and restoration of surface smoothness. Additionally, ultra-weak luminescence detection was conducted on the rats (Fig. 2C). The results revealed that the high-dose group ($p < 0.01$) effectively reversed the UV-induced superoxide burst in the skin, with photon intensity recovery reaching the level of the blank group (Fig. 2D,F), indicating significant anti-oxidative damage activity.

Histopathological Evaluation

H&E staining (Fig. 3A) was used to observe the overall histological structure of the skin, including epidermal thickness and general dermal morphology. The results revealed that the high-dose As-Gp group significantly inhibited UVA + UVB-induced abnormal epidermal hyperplasia. Compared with the model group, the dermis of As-Gp-treated rats appeared more organized, with reduced intercellular spaces and more homogeneous eosinophilic staining, suggesting improvement in extracellular matrix content. However, because H&E staining does not specifically distinguish collagen fibers from other matrix components, the detailed assessment of collagen fiber arrangement and density was based on complementary biochemical assays

(HYP, MMP-1/3) and aldehyde fuchsin staining for elastic fibers. Aldehyde fuchsin staining (Fig. 3B) was dedicated to the morphological evaluation of skin elastic fibers, and the results demonstrated that As-Gp promoted the functional recovery of skin's elastic mechanical properties by repairing the damaged elastic fiber network and inhibiting elastic fiber degeneration and fragmentation. Histological evidence confirmed that Acanthopanax senticosus glycoprotein reversed the pathological processes of photoaging through dual regulation of extracellular matrix (collagen and elastic fiber) remodeling and antioxidant stress pathways.

To evaluate the safety of As-Gp, histopathological analysis of major organs (heart, liver, spleen, lung, and kidney) was performed. H&E-stained images showed that the structures of all organs remained intact, with no obvious damage observed (Fig. 3L). These results indicate that As-Gp does not cause detectable toxicity or adverse effects in major organs under the current administration regimen, confirming its good biocompatibility.

Effect of As-Gp on the Expression of MDA, SOD, and GSH-Px in Rat Skin Tissue

SOD, GSH-Px, and MDA play critical roles in the antioxidant defense network [38,39]. MDA, a primary product of lipid peroxidation, reflects the degree of skin photoaging under oxidative stress, with elevated levels indi-

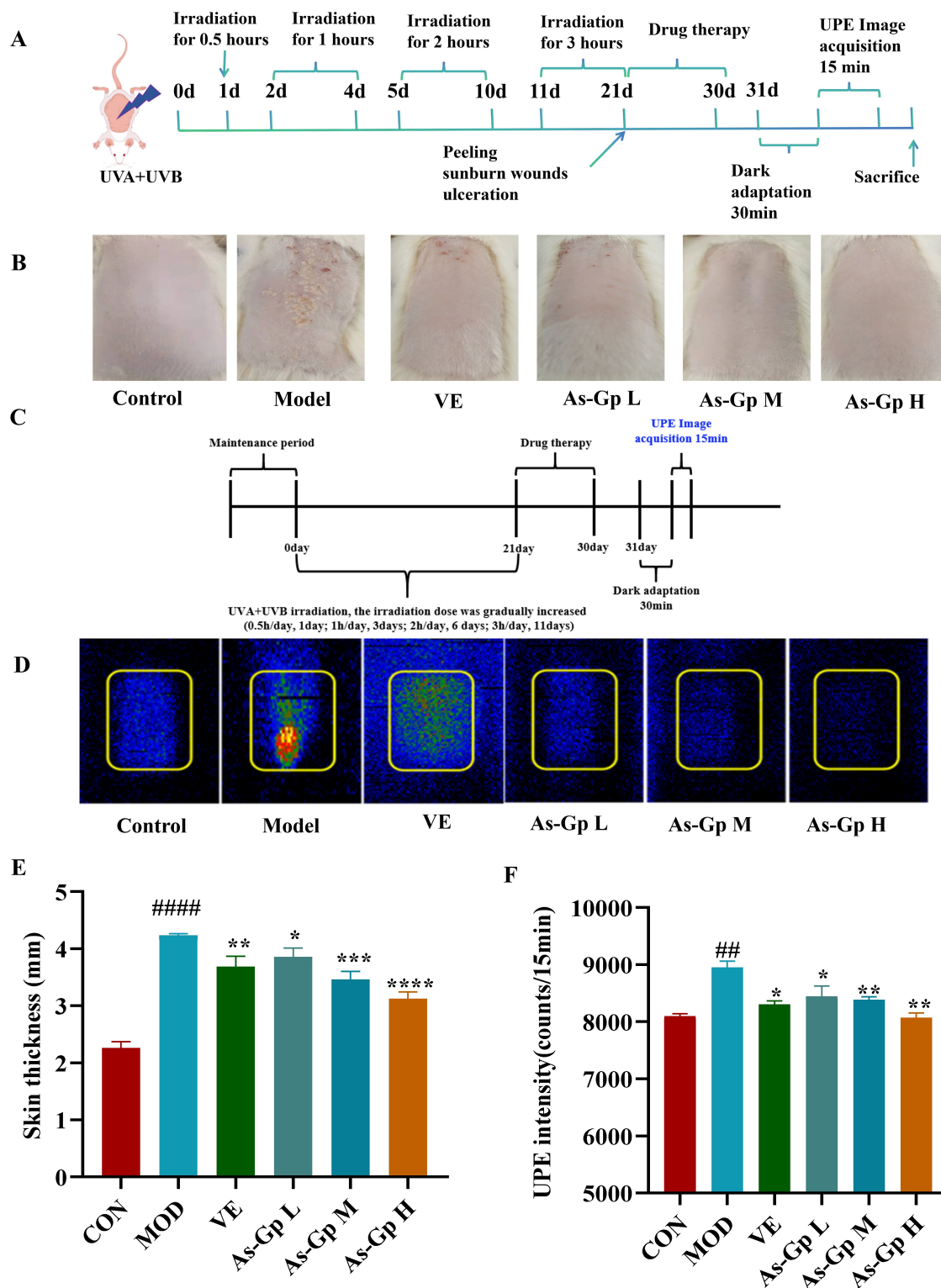


Fig. 2. Repair effects of Acanthopanax senticosusglycoprotein (As-Gp) on skin photoaging in rats. (A) Timeline of animal experiments. (B) Skin phenotype reconstruction. (C) Flowchart of dynamic ultra-weak luminescence monitoring. (D) Dynamic ultra-weak luminescence monitoring of skin oxidative stress status. (E) Quantitative analysis of dermal thickness. (F) Quantitative analysis of skin oxidative stress status by dynamic ultra-weak luminescence monitoring (Data are presented with statistical significance: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ N.S. indicates no statistical significance; # denotes significance between the model group and blank group; * denotes significance between the model group and treatment groups). Abbreviations: As-Gp, Acanthopanax senticosus glycoprotein; UPE, ultra-weak photon emission; UVA, long-wave UV; UVB, medium-wave ultraviolet.

cating exacerbated damage [40]. Experimental evidence confirms that As-Gp reverses the UV-induced photoaging process by activating the skin's antioxidant enzyme system. The MDA level in the model group was significantly higher than that in the blank group, whereas the high-dose As-Gp group (20 mg/mL, $p < 0.05$) showed a marked reduction to physiological levels, demonstrating its potent inhibitory effect on lipid peroxidation (Fig. 3C). Additionally, SOD and GSH-Px activities are key indicators for assessing skin oxidative damage following UV exposure [41]. ELISA assays revealed that As-Gp dose-dependently restored SOD activity, with the high-dose group ($p < 0.001$) exhibiting the most significant increase in SOD levels in rat skin tissue, closely approaching the levels seen in the blank group (Fig. 3D). In the VE-treated and low-/medium-/high-dose As-Gp groups, GSH-Px enzyme activity in rat skin tissue was restored to varying degrees, with the medium- and high-dose As-Gp groups showing a more pronounced increase ($p < 0.01$) (Fig. 3E). These data indicate that As-Gp intervenes in the pathological process of photoaging through a dual regulatory mechanism: synergistically activating the endogenous antioxidant defense network (SOD/GSH-Px) and inhibiting MDA production.

The Effect of As-Gp on the Expression of MMPs and HYP

Collagen degradation and elastin fiber fragmentation are among the most prominent features of skin photoaging [42]. Studies have shown that MMP-1 degrades collagen and collagen type III, while matrix metalloproteinase-3 (MMP-3) degrades cutaneous elastin, leading to the breakdown of collagen and elastin [43]. Therefore, this study measured the levels of MMP-1 and MMP-3 in rat skin tissue to evaluate the therapeutic effect of As-Gp on skin photoaging. The expression levels of MMP-1/3 in the UV model group were significantly higher than those in the blank group ($p < 0.0001$). After treatment, the MMP-1/3 levels in the skin tissue of rats in the VE group and the low-, medium-, and high-dose As-Gp groups were all reduced (Fig. 3F). Notably, the reduction in MMP-3 levels was particularly significant in the low- and medium-dose As-Gp groups ($p < 0.01$) (Fig. 3G). These findings suggest that As-Gp can effectively restore the collagen synthesis capacity of photo-damaged skin.

HYP, an important component with relatively high abundance among the 17 amino acids of collagen, plays a critical role in facilitating collagen reconstruction, elastin regeneration, and renewal *in vivo*. Therefore, the HYP content in rat skin tissue serves as a biomarker amino acid that directly reflects the collagen content in the skin [44]. Assays of this key collagen biomarker (HYP) revealed that the medium- and high-dose As-Gp groups significantly restored skin collagen synthesis ($p < 0.01$), with their collagen regeneration-promoting efficacy being more pronounced compared to the VE group (Fig. 3H). These results indicate that As-Gp can effectively restore the col-

lagen synthesis capacity of photo-damaged skin.

Effect of As-Gp on the Expression of IL-6, IL-1 β , and TNF- α

Excessive UV radiation irradiation of the skin induces the overexpression of inflammatory cytokines (e.g., IL-6, IL-1 β) and tumor necrosis factor (TNF- α) in epidermal and dermal cells, triggering inflammatory responses and the development of related skin diseases [45]. As-Gp significantly inhibited the activation of the UV-induced skin inflammatory network. The levels of IL-6 ($p < 0.05$), IL-1 β ($p < 0.0001$), and TNF- α ($p < 0.01$) in the model group were significantly higher than those in the blank group. The high-dose As-Gp group exhibited a notably stronger inhibitory effect on IL-1 β ($p < 0.05$) compared to the VE group (Fig. 3I,J). TNF- α detection revealed that As-Gp displayed a dose-dependent regulatory advantage: the high-dose group restored TNF- α levels to the baseline of the blank group, showing a significant improvement compared to the VE group ($p < 0.01$) (Fig. 3K).

As-Gp constitutes a triple synergistic anti-photoaging mechanism by specifically inhibiting the excessive release of key inflammatory cytokines (IL-1 β /TNF- α), blocking the UV-induced skin inflammatory cascade, and interacting with previously identified antioxidant and matrix remodeling mechanisms.

As-Gp Promotes HaCaT Cell Proliferation and Migration

The MTT assay results revealed that As-Gp exhibited a concentration-dependent bidirectional regulatory effect on HaCaT cell proliferation. Within the concentration range of 10–100 $\mu\text{g/mL}$, the cell proliferation rate significantly increased with rising concentrations (from 61% to 85%), with the 100 $\mu\text{g/mL}$ group showing the peak proliferative effect (85%) ($p < 0.0001$). These findings indicate that As-Gp promotes keratinocyte proliferation (Fig. 4A).

Cell migration is a core process for epidermal repair after photodamage, and this study evaluated the migratory capacity of HaCaT cells treated with As-Gp through two-dimensional scratch assay and three-dimensional Transwell assay, respectively. Scratch assay results (Fig. 4B) demonstrated that As-Gp significantly enhanced the two-dimensional migratory capacity of HaCaT cells in a dose-dependent manner. Observations at 24 hours revealed that the scratch wound closure rate in the high-concentration As-Gp group (50 $\mu\text{g/mL}$) was significantly higher than that in the low-concentration groups and the blank control group, confirming that As-Gp accelerates epidermal wound healing by promoting keratinocyte migration on the planar level.

Transwell assay further verified the regulatory effect of As-Gp on HaCaT cell migration in the three-dimensional microenvironment (Fig. 4I). Results showed that As-Gp at low, medium, and high concentrations all promoted HaCaT cell transmembrane migration; the medium-concentration

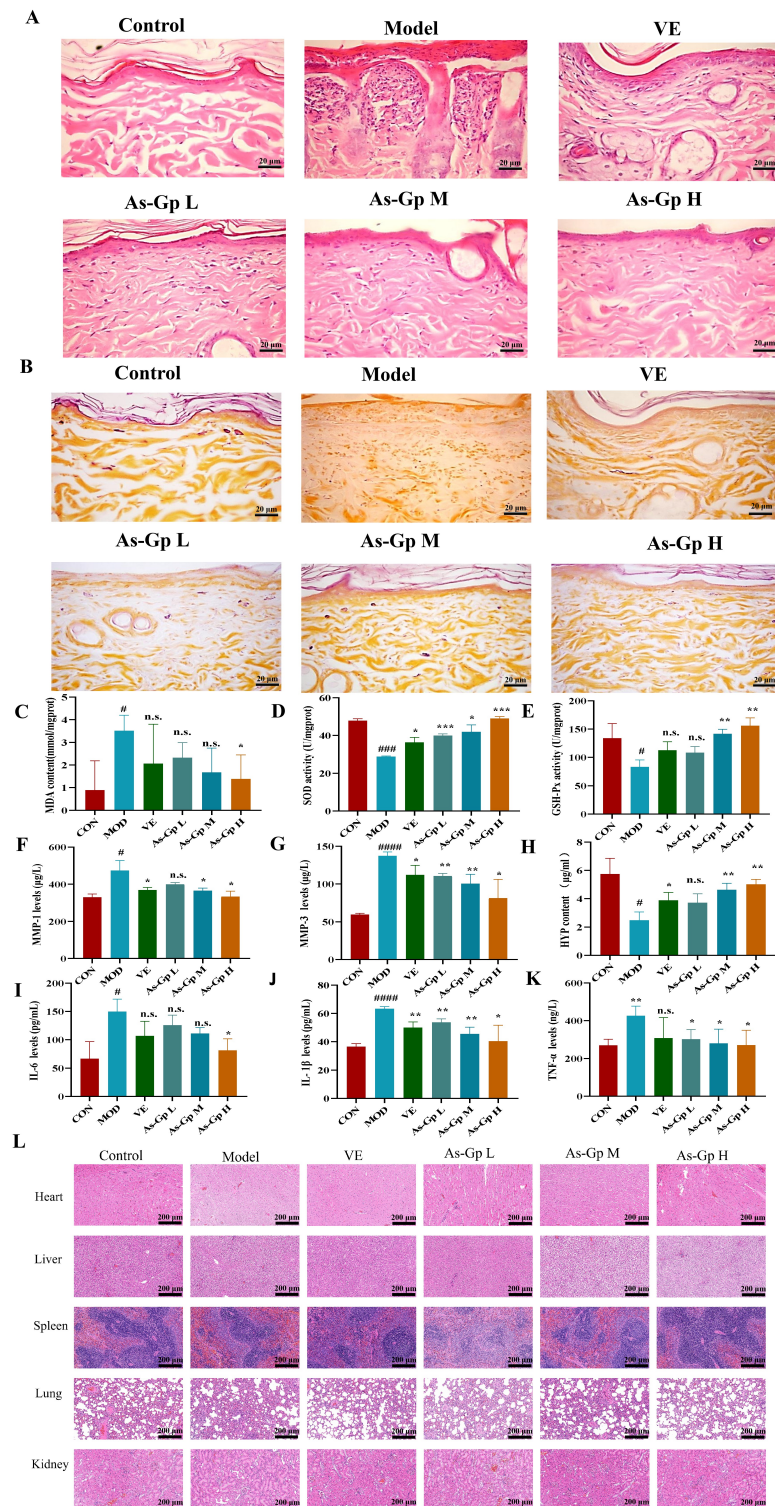


Fig. 3. Therapeutic effects of As-Gp on UV-induced skin photoaging in rats. (A) Rat skin pathological sections, H&E staining. (Scale bar: 20 μm). (B) Aldehyde fuchsin staining of rat skin pathological sections. (Scale bar: 20 μm). (C, D, E) Quantitative analysis of oxidative stress markers (MDA, SOD, GSH-Px). (F, G) Expression regulation of matrix-degrading enzymes (MMP-1/3). (H) Detection of the collagen synthesis marker HYP. (I, J, K) Cascade inhibition of inflammatory cytokines (IL-6, IL-1β, TNF-α); (L) H&E staining of the heart, liver, spleen, lung, and kidney. (Scale bar: 200 μm) (Data are presented with statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. N.S. indicates no statistical significance; # denotes significance between the model group and blank group; * denotes significance between the model group and treatment groups. Abbreviations: As-Gp, Acanthopanax senticosus glycoprotein; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; HYP, hydroxyproline; IL-1β, interleukin-1β; MMP, matrix metalloproteinase; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

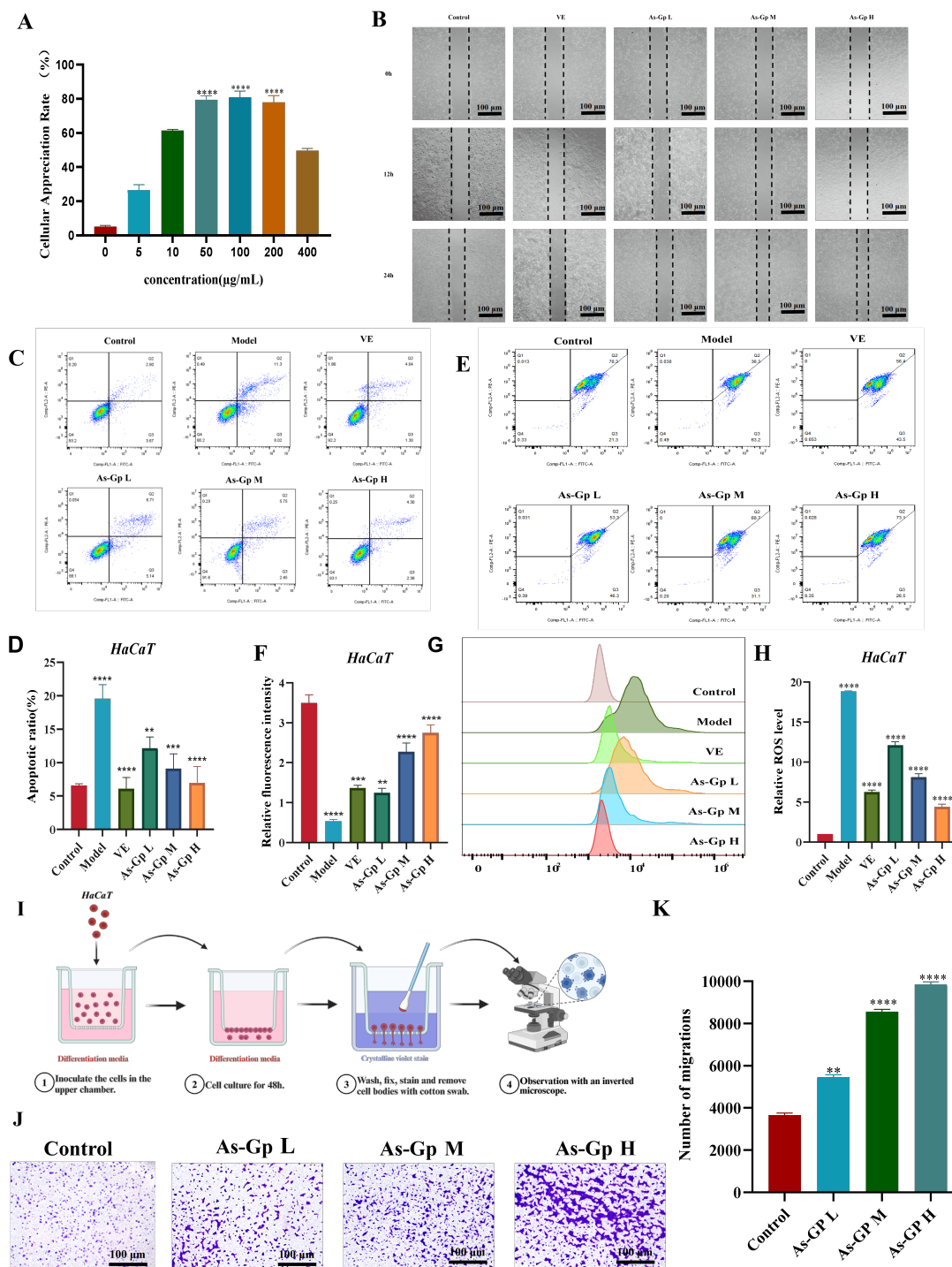
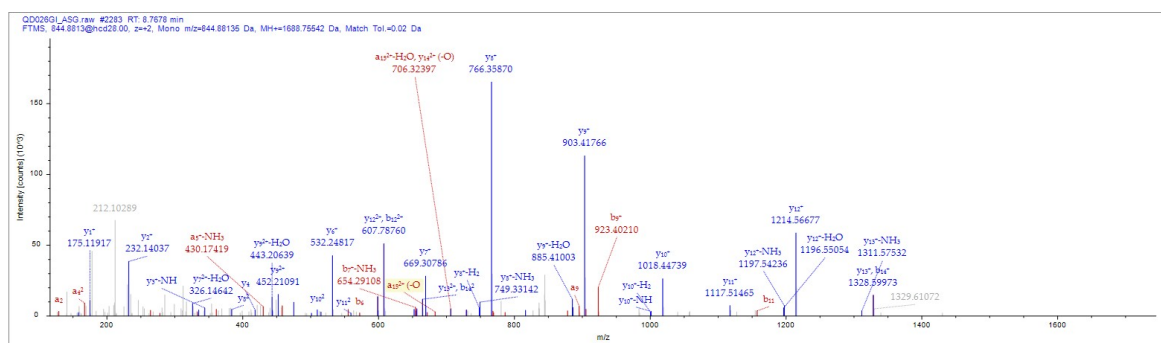
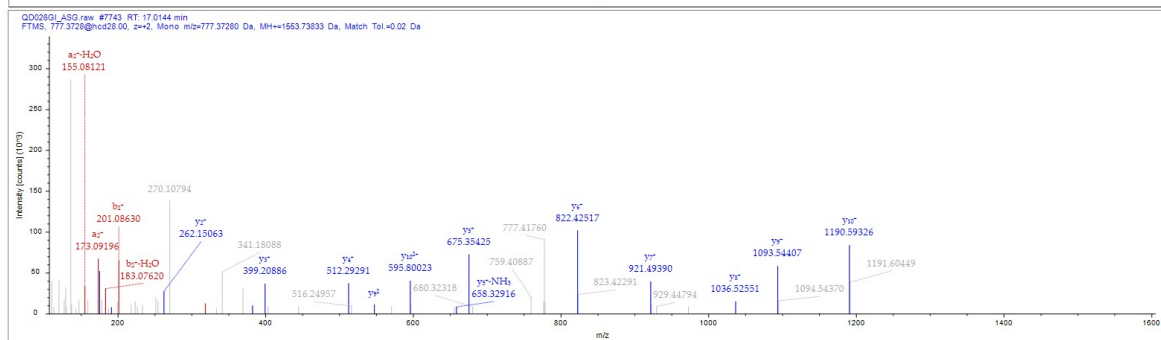


Fig. 4. Therapeutic effects of As-Gp on UV-induced skin photoaging. (A) Effect of As-Gp on HaCaT cell proliferation. (B) Effect of As-Gp on HaCaT cell scratch wound healing (Scale bar: 100 μm). (C) Effect of As-Gp on reducing HaCaT cell apoptosis. (D) Quantitative assessment of As-Gp's effect on reducing HaCaT cell apoptosis. (E) Effect of As-Gp on restoring mitochondrial membrane potential in HaCaT cells. (F) Quantitative assessment of As-Gp's effect on restoring mitochondrial membrane potential in HaCaT cells. (G) Effect of As-Gp on alleviating oxidative stress in HaCaT cells. (H) Quantitative assessment of As-Gp's effect on alleviating oxidative stress in HaCaT cells. (I) Schematic diagram of the Transwell assay. Created in biorender. Jia, X. (2025) <https://BioRender.com/wvwm-p2s>; (J) Effect of As-Gp on promoting HaCaT cell migration. (Scale bar: 100 μm). (K) Quantitative assessment of As-Gp's effect on promoting HaCaT cell migration. (Data are presented with statistical significance: ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. N.S. indicates no statistical significance; # denotes significance between the model group and blank group; * denotes significance between the model group and treatment groups. Abbreviations: As-Gp, Acanthopanax senticosus glycoprotein; UV, ultraviolet; HaCaT, human immortalized keratinocyte cell line; ROS, reactive oxygen species.

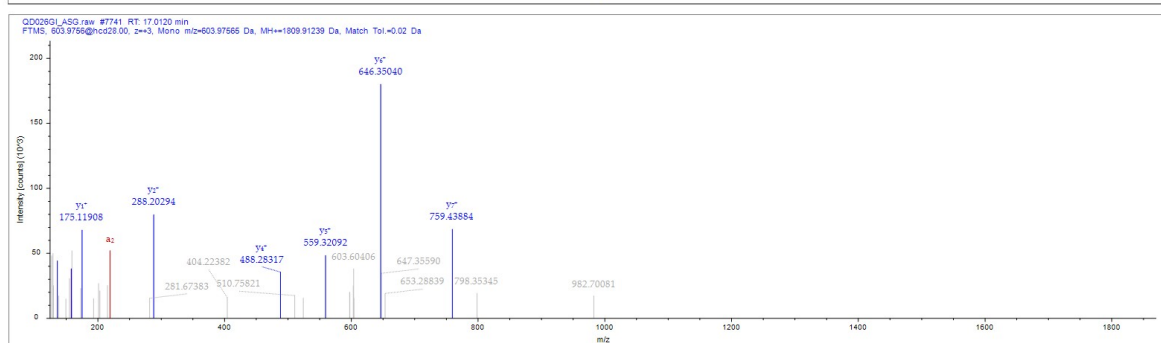
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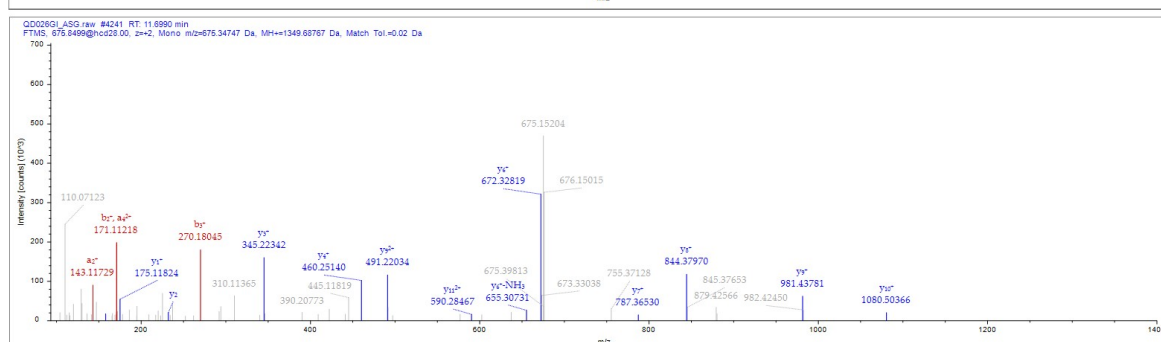
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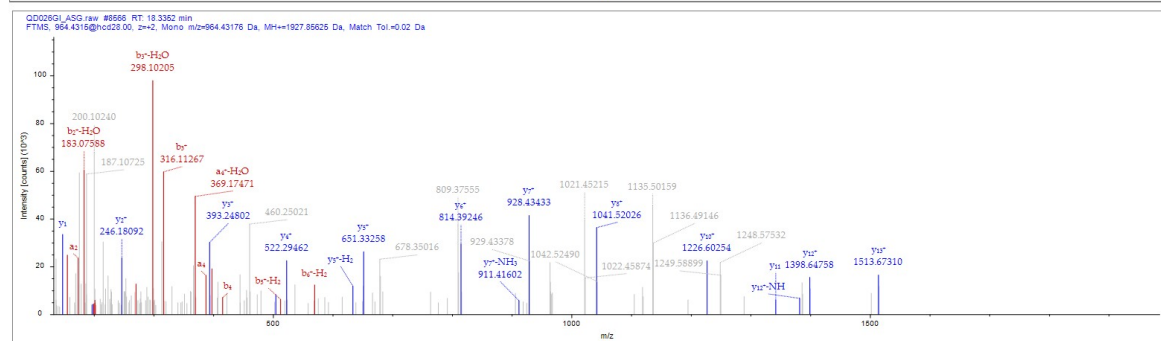


Fig. 5. Results of target protein analysis in *Acanthopanax senticosus* glycoprotein (As-Gp). (A) Mass spectrum of peptide GVGM-NPVDHPHGGGEGR. (B) Mass spectrum of peptide EAYPGDVFYLHSR. (C) Mass spectrum of peptide VFDKQNGFISAAELR. (D) Mass spectrum of peptide EADVDGDGQINYEYFVK. (E) Mass spectrum of peptide AVVVHGDPPDLLG.

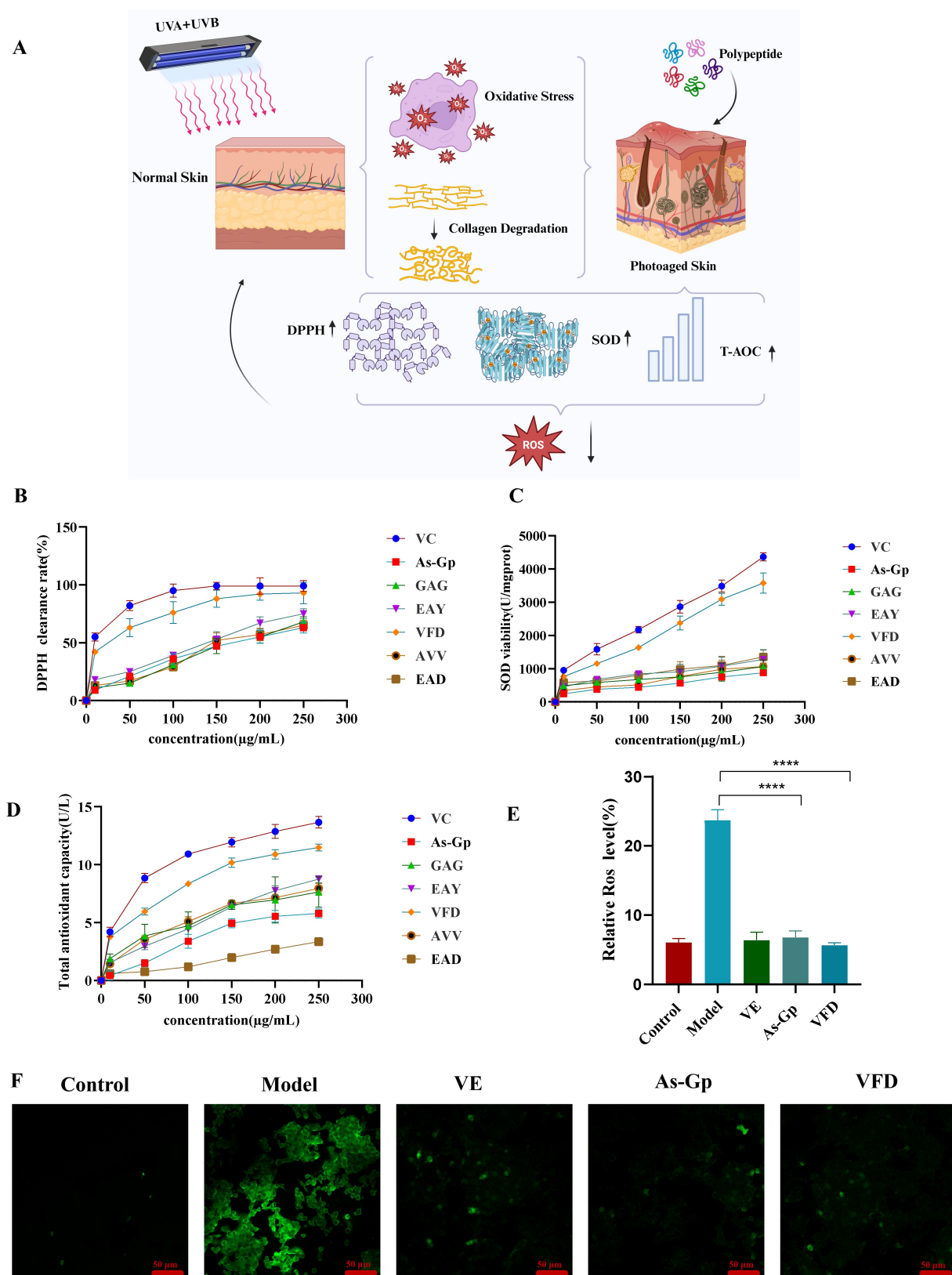


Fig. 6. Verification of antioxidant activity of synthetic peptides. (A) Schematic diagram of the antioxidant pathway of synthetic peptides, Created in biorender (2025) <https://BioRender.com/ky2hcs0>; (B) Comparison of DPPH radical scavenging rates. (C) Comparison of SOD activity. (D) Comparison of total antioxidant capacity (T-AOC). (E,F) Confocal immunofluorescence images of reactive oxygen species (ROS) (Scale bar: 50 μ m). **** $p < 0.0001$. Abbreviations: VC, vitamin C; VE, vitamin E; As-Gp, Acanthopanax senticosus glycoprotein; VFD, peptide VFDKDQNGFISAAELR; GVG, peptide GVGMNPVDHPHGGGEGR; EAY, peptide EAYPGDVLYLHSR; AVV, peptide AVVVHGDPPDLG; EAD, peptide EADVGDGQINYEYFVK.

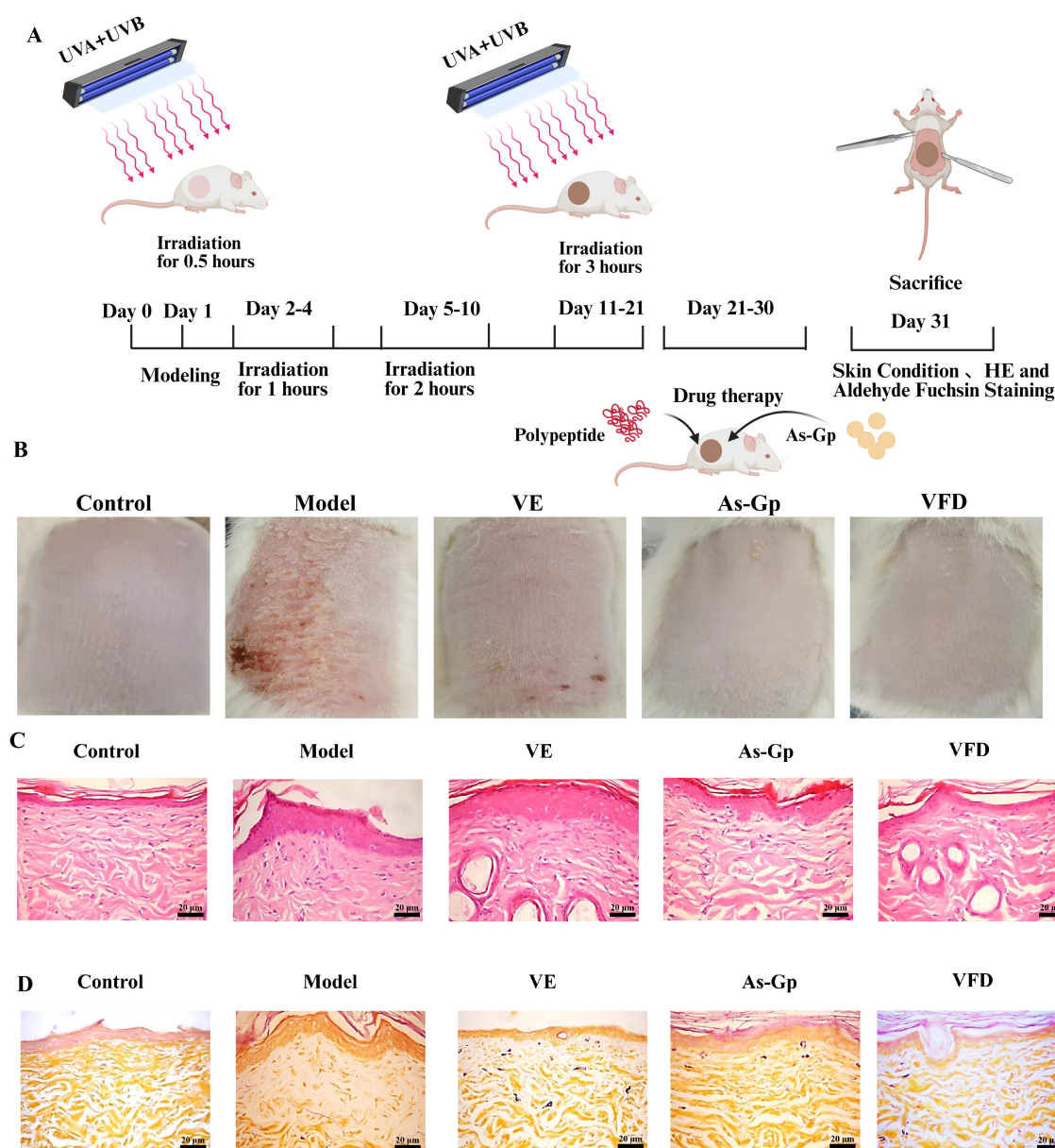


Fig. 7. Repair effects of Acanthopanax senticosus glycoprotein (As-Gp) and VFD on skin photoaging in rats. (A) Timeline of animal experiments, Created in biorender (2025) <https://BioRender.com/b06f9l2>. (B) Skin phenotype reconstruction. (C) Rat skin pathological sections HE staining (Scale bar: 20 μ m). (D) Aldehyde fuchsin staining of rat skin pathological sections (Scale bar: 20 μ m). Abbreviations: As-Gp, Acanthopanax senticosus glycoprotein; VFD, peptide VFDKQNGFISAAELR; VE, vitamin E; UV, ultraviolet.

group exhibited a moderate enhancement, while the high-concentration group showed the most significant increase in the number of migrated cells (Fig. 4J,K). Collectively, the scratch and Transwell assays consistently confirmed that As-Gp can significantly promote HaCaT cell migration in a dose-dependent manner, which is a key mechanism for As-Gp to accelerate the repair of UV-damaged skin epidermis.

As-Gp Reduces HaCaT Cell Apoptosis

Annexin V/PI double-staining flow cytometry analysis revealed that the apoptosis rate in the model group was significantly higher than in the control group ($p < 0.01$),

whereas the apoptosis rate in the drug intervention group decreased in a dose-dependent manner ($p < 0.01$), with the high-dose group exhibiting the most pronounced inhibitory effect (Fig. 4C,D). These results indicate that As-Gp effectively antagonizes UVA/UVB-induced apoptosis in HaCaT cells.

As-Gp Restores the Mitochondrial Membrane Potential Function of HaCaT Cells

A decline in mitochondrial membrane potential is a characteristic early event of apoptosis. JC-1 probe-based detection revealed that, in normal cells with intact mi-

tochondrial membrane potential, JC-1 aggregates within mitochondria to form red fluorescent polymers. In contrast, in apoptotic cells, membrane potential depolarization causes JC-1 release and dissociation into green fluorescent monomers. The red/green fluorescence intensity ratio thus reflects the status of mitochondrial membrane potential (Fig. 4E,F). Results showed that, compared to the control group, the proportion of mitochondrially depolarized cells in the model group was significantly increased ($p < 0.01$). Drug intervention groups (especially the high-dose group) dose-dependently increased the proportion of cells with intact mitochondrial membrane potential and enhanced the red/green fluorescence intensity ratio, indicating their ability to restore mitochondrial membrane potential.

As-Gp Alleviates Oxidative Stress in HaCaT Cells

DCFH-DA probe-based detection revealed that the ROS fluorescence intensity in the model group was significantly higher than that in the control group ($p < 0.01$), whereas ROS production in the drug intervention groups decreased in a dose-dependent manner ($p < 0.01$) (Fig. 4G,H). These results suggest that As-Gp may alleviate phototoxic damage by reducing UVA/UVB-induced ROS accumulation in HaCaT cells.

The Effective Concentration Range and the Optimal Dose of As-Gp

The comprehensive analysis of *in vitro* and *in vivo* experimental results determined the effective concentration range and optimal dosage of As-Gp in the repair of UV-induced skin photoaging, and based on empirical data, provided different parameters. The effective concentration range for cell proliferation is 10–100 $\mu\text{g/mL}$, with the optimal dose being 100 $\mu\text{g/mL}$ (proliferation rate can reach up to 85%, $p < 0.0001$); for cell migration (scratch and Transwell assays), the effective range is 10–50 $\mu\text{g/mL}$, with the optimal dose being 50 $\mu\text{g/mL}$ (significantly enhancing wound closure and transmembrane migration); for anti-apoptosis, mitochondrial membrane potential restoration, and ROS clearance, the effective concentration range is stable at 50–200 $\mu\text{g/mL}$, with the optimal dose being 200 $\mu\text{g/mL}$ (most significant inhibitory effects on apoptosis and ROS accumulation, $p < 0.001$). When the concentration exceeds 200 $\mu\text{g/mL}$, there is no further enhancement in biological activity in the proliferation assay, and even slight cytotoxicity may occur. The effective concentration range of As-Gp is 5–20 mg/mL. The low-dose group (5 mg/mL) showed a trend of improving the skin photoaging phenotype, but with no statistically significant difference compared to the model group; the medium-dose group (10 mg/mL) demonstrated significant therapeutic effects on oxidative stress and matrix metabolism ($p < 0.01$); the high-dose group (20 mg/mL) achieved the most comprehensive repair effects, including significant reduction in skin thickness, restoration of collagen/elastin fiber structure, inhi-

bition of inflammatory cytokine release (IL-1 β , TNF- α), and restoration of oxidative stress markers (SOD, GSH-Px, MDA) to near normal levels ($p < 0.001$ – 0.0001). Therefore, the optimal *in vivo* dosage was determined to be 20 mg/mL.

Overall, As-Gp exhibited a concentration-dependent therapeutic effect in UV-induced skin photoaging. The effective concentration range and optimal dosage varied depending on the experimental system, providing a key foundation for its translational application.

Identification of the Target Protein in As-Gp

After clarifying the role of As-Gp in photoaged skin, confirming its protein structure, and identifying the target proteins of the drug are crucial for understanding its mechanism of action and potential applications. This study combined in-gel digestion with high-resolution mass spectrometry (MS) (Supplementary Fig. 3) to analyze the target proteins in As-Gp, and performed data analysis using Proteome Discoverer 2.4. Four characteristic functional proteins were identified in the 25 kDa fraction of As-Gp: ribosomal protein Rpl2, ATPase Atp, calmodulin (Cam), and SOD homologous protein (Supplementary Table 2). MS spectra (Fig. 5A–E) confirmed five characteristic peptide sequences (e.g., GVGMPVDHPHGGGEGR and EAYPGDVFYLSHR), completing the construction of the molecular fingerprint map of the active component (Supplementary Table 3).

In vitro Synthesis and Identification of the Target Peptide in As-Gp

The target protein in As-Gp was identified, followed by biosynthesis using biotechnological methods, with the synthesis protocol detailed in Supplementary Table 1. After synthesis, *in vitro* antioxidant activity was evaluated to identify the key peptides or proteins responsible for the bioactivity. Upon successful synthesis, peptides 1–5 were tested and characterized, and their basic physicochemical properties were summarized, with the results presented in Table 3.

To ensure the quality of the *in vitro* synthesized peptides, high-performance liquid chromatography (HPLC) was used to analyze the five peptides. The HPLC analysis showed that the five peptides exhibited stable retention times (6.742–12.130 min), with peak areas accounting for over 95% and a relative standard deviation (RSD) of less than 0.5% ($n = 3$), meeting the pharmaceutical-grade quality standards (Supplementary Fig. 4–Supplementary Fig. 8).

Additionally, through MS analysis, we detected the molecular weight and structural information of the *in vitro* synthesized peptides. ESI-MS/MS confirmed the target molecular ion peaks (m/z 558.95–1350.94), with the degree of matching for secondary fragment ions exceeding 95%. Specifically, peptide 3 (VFDKQNG-

Table 3. Physicochemical properties of synthetic peptides.

Polypeptide physical properties	Polypeptide1	Polypeptide2	Polypeptide3	Polypeptide4	Polypeptide5
State	white powder	white powder	white powder	white powder	white powder
Purity	96.79%	96.84%	96.36%	97.79%	96.09%
Molecular weight	1672.81	1553.70	1810.00	1349.47	1928.70
Solubility	1 mg/mL in 15% ACN/85% H ₂ O	1 mg/mL in 17% ACN/83% H ₂ O	1 mg/mL in 17% ACN/83% H ₂ O	1 mg/mL in 25% ACN/75% H ₂ O	1 mg/mL in 33% ACN/67% H ₂ O

Abbreviations: ACN, acetonitrile.

FISAAELR) exhibited a characteristic fragmentation pattern at m/z 604.72/906.41, with a deviation from the theoretical molecular weight of < 0.01% (**Supplementary Fig. 9–Supplementary Fig. 13**).

Verification and Comparison of in vitro Antioxidant Activity Between As-Gp and Synthetic Peptides

Free radical-induced oxidative stress injury is considered a key pathogenic mechanism for various chronic health issues [46]. Studies have shown that UVA, with strong penetrating power, can deeply reach the dermis layer of the skin, induce the production of ROS, and thereby trigger oxidative stress reactions [47]; UVB primarily affects superficial skin cells, leading to abnormal formation of free radicals and oxidative damage [48]. Vitamin C (VC) (ascorbic acid) and VE (α -tocopherol) were selected as positive controls in different antioxidant experimental systems based on their distinct antioxidant properties: VC is a water-soluble antioxidant that directly scavenges free radicals in the aqueous phase and is used for *in vitro* chemical antioxidant activity assessment; VE is a lipid-soluble antioxidant that primarily inhibits lipid peroxidation in biological membranes, making it more suitable for cell and *in vivo* anti-photoaging experiments. In this section, VC serves as a positive control in *in vitro* chemical experiments (DPPH, SOD, T-AOC) to evaluate the direct free radical scavenging and antioxidant capacity of As-Gp and synthesized peptides. In this section, VC was used as the positive control for *in vitro* chemical assays (DPPH, SOD, total antioxidant capacity (T-AOC)) to evaluate the direct free radical-scavenging and antioxidant capacity of As-Gp and synthetic peptides.

To verify the antioxidant activity of As-Gp and five synthetic peptides (GVG, EAY, VFD, AVV, EAD), this study evaluated them through three methods: DPPH radical scavenging capacity [49], SOD activity [50], and T-AOC [51]. The antioxidant mechanism diagram is shown in Fig 6A.

The DPPH radical scavenging assay (Fig. 6B) demonstrated that the VFD peptide exhibited significantly higher activity compared to other synthetic peptides, second only to the positive control, VC. The T-AOC, measured by the ferric reducing antioxidant power (FRAP) assay (Fig. 6C), revealed the following activity ranking for the VFD peptide: VC > VFD > EAY > AVV > GVG > As-Gp > EAD (p <

0.01). Further confirmation from the SOD enzyme activity assay (Fig. 6D) showed that within the concentration range of 0–0.25 mg/mL, the VFD peptide had the strongest activating effect on SOD activity (p < 0.05), with the following activity ranking: VC > VFD > EAD > EAY > AVV > GVG > As-Gp. Concurrently, immunofluorescence analysis indicated that ROS fluorescence intensity was significantly higher in the model group compared to the blank control, while ROS production in the drug intervention groups decreased in a dose-dependent manner. Notably, the synthetic VFD peptide group exhibited effects comparable to those of the VE group (Fig. 6E,6F).

Experimental results indicated that the VFD peptide is the core antioxidant component of the As-Gp. Its DPPH radical scavenging activity, T-AOC, and SOD activation effect were all significantly superior to those of other synthetic peptides, providing critical experimental evidence for subsequent functional validation and application research.

Comparison of the in vivo Anti-Photoaging Effects of Synthetic Peptides and As-Gp

The *in vitro* antioxidant experiment indicates that the VFD peptide is the core antioxidant component of As-Gp. Based on this, we further compared the *in vivo* anti-photoaging effects of synthetic peptide VFD and As-Gp. After successful model establishment, the skin changes of the rats in each group were photographed and recorded during the drug administration period, including skin thickness and recovery status (Fig. 7A). On the 7th day of administration, significant improvement in skin phenotype was observed in all groups (Fig. 7B). The model group exhibited typical photoaging characteristics (leathery thickening with epidermal damage), whereas the VE, As-Gp, and VFD peptide groups achieved epidermal structure reconstruction, characterized by a significant reduction in skin thickness, hair regrowth, and restored surface smoothness.

H&E staining (Fig. 7C) was used to evaluate epidermal thickness and general dermal architecture. The results showed that the VFD and As-Gp groups significantly inhibited UVA + UVB-induced epidermal hyperplasia. The dermis in these groups appeared more compact and uniformly stained compared to the model group, indicating improvement in overall extracellular matrix organization. Specific assessment of collagen fiber remodeling was sup-

ported by biochemical markers (HYP, MMP-1/3) and aldehyde fuchsin staining of elastic fibers; the effect of the VFD group was comparable to that of the VE group. Aldehyde fuchsin staining (Fig. 7D) was used for the specific evaluation of elastic fiber repair, and the results showed that these components restored skin elastic mechanical properties by repairing the damaged elastic fiber network and inhibiting matrix degeneration. Combined with the previous detection of collagen-related biochemical indexes (HYP, MMP-1/3), histological evidence further confirmed that the VFD peptide, as the core antioxidant component of As-Gp, reverses the pathological process of photoaging through dual regulation of extracellular matrix (collagen and elastic fiber) synthesis and oxidative stress pathways.

Discussion

This study preliminarily clarified the mechanism of *Acanthopanax senticosus* glycoprotein (As-Gp) in repairing UV-induced skin photoaging through *in vivo* and *in vitro* experiments, and identified the core peptide segment VFDKDQNGFISAAELR-(Cam) with antioxidant activity. Based on the current experimental results, the anti-photoaging effect of As-Gp may involve synergistic regulation across multiple pathways.

UV irradiation first causes mitochondrial dysfunction in skin cells, manifested as increased ROS levels and decreased mitochondrial membrane potential [52,53]. After As-Gp treatment, it dose-dependently upregulates the activities of SOD and GSH-Px, reduces MDA content, and decreases intracellular ROS accumulation. Furthermore, As-Gp also exerts inhibitory effects on two downstream pathways: first, it inhibits the release of inflammatory factors IL-1 β , TNF- α , and IL-6; second, it downregulates the expression of MMP-1/3, reduces collagen degradation, and promotes HYP synthesis, which benefits the restoration of the extracellular matrix structure. In addition, As-Gp promotes HaCaT cell proliferation and migration, accelerating the repair of damaged epidermis. Overall, the effects of As-Gp are not limited to a single target, but involve multiple processes including oxidative stress regulation, inflammation suppression, matrix metabolism regulation, and tissue repair.

Compared to other natural anti-photoaging ingredients reported in the literature, As-Gp and its core peptide VFD have distinct characteristics in terms of activity level and mechanism of action [54–56]. In *in vitro* antioxidant experiments, the VFD peptide's ability to scavenge DPPH radicals is comparable to that of VC (IC₅₀ = 18.23 μ g/mL), outperforming the reported values of sesame glycoproteins and some polyphenolic compounds. In SOD activity and T-AOC tests, the performance of VFD peptide also surpassed that of the crude extract of As-Gp and four other synthetic peptides. Unlike retinol derivatives or chemical sunscreens, which target relatively single pathways, As-Gp has antioxidant, anti-inflammatory, and repair-promoting functions.

It not only inhibits MMPs expression but also reduces oxidative damage at its source while promoting collagen synthesis. Compared to novel biological agents like stem cell exosomes, As-Gp, as a plant-derived natural product, has certain advantages in terms of preparation cost, storage stability, and safety.

This study still has some limitations that need to be addressed in future work. Although the core peptide segment VFD with antioxidant activity was selected from As-Gp, the biological functions of the other four characteristic peptides (GVG, EAY, AVV, EAD) and the glycan portion remain unclear. Whether the glycosylation modifications in the glycoprotein affect the stability, bioavailability, or binding capacity of the peptide segments with their targets requires further investigation. While this study confirmed that As-Gp can regulate mitochondrial function and inflammatory responses, the specific molecular targets remain undefined. For example, it is still unclear whether the VFD peptide directly binds to calmodulin (Cam) or which downstream signaling molecules it acts upon. Future research can utilize techniques such as surface plasmon resonance (SPR) and drug affinity responsive target stability (DARTS) for target capture and verification. This study employed UPE technology to achieve non-invasive, real-time monitoring of skin oxidative stress, but the correlation between photon signals and specific molecular events (such as certain ROS species or lipid peroxidation products) still needs to be clarified and verified in subsequent research.

Conclusions

This study systematically and comprehensively elucidates the multi-target synergistic mechanism of As-Gp in the repair of UV-induced skin photoaging, demonstrating significant innovation. Methodologically, the study pioneers the application of UPE technology in photoaging research, achieving non-invasive, real-time dynamic monitoring of skin oxidative stress recovery after UV radiation, overcoming the limitations of traditional static detection. Mechanistically, As-Gp inhibits oxidative stress by upregulating SOD/GSH-Px activity and reducing MDA/ROS levels, alleviates inflammation by inhibiting the release of IL-1 β /TNF- α /IL-6, promotes HaCaT cell proliferation and migration, accelerates epidermal repair, and restores mitochondrial membrane potential to maintain cellular homeostasis, thereby filling the gap in understanding the anti-photoaging effect of As-Gp. In terms of components, proteomics analysis identified four functional proteins in the 25 kDa active component of As-Gp, and successfully screened five characteristic peptide chains. Among them, VFDKDQNGFISAAELR-(Cam) was verified as the core antioxidant peptide, exhibiting DPPH radical scavenging activity and SOD activation effects comparable to those of VC. In conclusion, As-Gp, as a natural skin photoaging repair agent, demonstrates multi-target efficacy and high safety, promoting the development and utilization of *Acan-*

thopanax resources, and providing new ideas and technical support for the translational application of phytopharmacology in the field of dermatology.

List of Abbreviations

As-Gp, *Acanthopanax senticosus* glycoprotein; ROS, reactive oxygen species; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; IL-1 β , interleukin-1 β ; MMP, matrix metalloproteinase; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; HYP, hydroxyproline; UPE, ultra-weak photon emission; UV, ultraviolet; HaCaT, human immortalized keratinocyte cell line; VFD, peptide VFDKDQNGFISAAELR; GVG, peptide GVGMPVDHPHGGGEGR; EAY, peptide EAYPGDVFYLSHR; AVV, peptide AVVVHGDPPDDL; EAD, peptide EADVGDGQINYEFEVK; UVC, short-wave ultraviolet; UVB, medium-wave ultraviolet; UVA, long-wave ultraviolet; PABA, p-aminobenzoic acid; TCM, Traditional Chinese Medicine; MMP-1, matrix metalloproteinase-1; SPF, specific pathogen-free; SD, Sprague-Dawley; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; O-GlcNAc, O-linked N-acetylglucosamine modification; BCA, bicinchoninic acid; IR, Infrared; TFA, trifluoroacetic acid; GC, gas chromatography; H&E, hematoxylin-eosin; OD, optical density; SD, standard deviation; ANOVA, analysis of variance; MMP-3, matrix metalloproteinase-3; MS, mass spectrometry; HPLC, high-performance liquid chromatography; RSD, relative standard deviation; VC, vitamin C; T-AOC, total antioxidant capacity; SPR, surface plasmon resonance; DARTS, drug affinity responsive target stability; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; O-GlcNAc, O-linked N-acetylglucosamine modification; MED, minimal erythema dose; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; PI, propidium iodide; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; VE, vitamin E; JC-1, 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide; ACN, acetonitrile.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. Additional raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization, YJ and DMF; methodology, ZYY, YJ. And XSJ; software, XSJ; validation, DMF, and ZYY; formal analysis, JH; investigation, DL; resources, YYJ; data curation, JH; writing—original draft preparation,

DMF; writing—review and editing, HLW and JMX; visualization, HLW and JMX; supervision, DL; project administration, HLW; funding acquisition, DL. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

The animal study protocol was approved by the Ethics Committee of Changchun University of Chinese Medicine (Approval No 2025042, Qualification Certificate Number: SCXK(Ji)-2020-0002).

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Conflict of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.22203/eCM.v059a06>.

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